IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
Daryn KENNY et al.

Confirmation No.: 9458

Serial No.: 09/872,493

Group Art Unit: 1634

Filing Date: June 1, 2001

Examiner: Jeanine Anne GOLDBERG

Title: HIGHLY SENSITIVE GENE DETECTION AND LOCALIZATION USING IN SITU BRANCHED-DNA HYBRIDIZATION

DECLARATION UNDER 37 C.F.R. § 1.131
INVENTOR DARYN KENNY

I, Daryn Kenny, declare:

1. I am an inventor of the patent application identified above. At the time of the present invention I was employed by Bayer Corporation and I am still employed by Bayer Corporation.

2. I have read the Antao et al. reference and fully understand its contents.

3. I have been told by my patent attorney, Karen Canaan, that because the publication date of the Antao et al. reference is January 21, 2000, a date less than one year before the June 2, 2000, filing date of this application's priority provisional application, the Antao et al. reference may be eliminated as prior art against the claimed invention if the date of the present invention predates the publication date of the Antao et al. reference.

4. Attached to this Declaration are six laboratory notebook pages from my laboratory notebook for this invention that were signed by me and witnessed at Bayer Corporation on a date that predates the January 21, 2000, publication date of Antao et al. Although the notebook pages have been redacted to hide the execution and witnessing dates, I am declaring herein that both the date of execution and the witnessing dates are before January 21, 2000.

5. The submitted pages document single copy gene detection of HPV-16 DNA in cell lines using bDNA *in situ* hybridization; the following inventive results are shown in the laboratory pages:

a. Pages 71 and 83 provide a summary of the results from the study. Page 71 describes how an impartial observer would be able to discern the positive results; specifically, that SiHa cells probed with bDNA probes are positive for HPV-16 DNA and negative for HPV-18 DNA.

b. Pages 71 and 73 document the protocol used to generate the results.

c. Pages 86 and 92 document single copy gene detection results. The dark ovals in the figures are cell nuclei and the dark specks or spots over the cell nuclei indicate the presence of HPV DNA. The SiHa cell line is a standard model for low HPV-16 copy number; detection of HPV-16 in SiHa cells demonstrates the sensitivity of the bDNA ISH technology. The CaSki cell line has hundreds of HPV-16 copies and the HeLa cell line has dozens of HPV-18 copies. The figures show three panels for each cell line that compares results with the HPV-16 and HPV-18 probes. The absence of spots over the SiHa cells for HPV-18 probes demonstrates the specificity of the bDNA ISH method.

6. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the instant application or any patent issuing thereon.

Dated: 2/25/05

Daryn Kenny

Daryn Kenny, Ph.D.

71

BAYER CORPORATION

SUBJECT bDNA in situ

Daryn Kenny

This week's bDNA assays reiterate the SiHa and HeLa sensitivity v. background issue. Three independent experiments were done, three separate days with four samples each cell lines comparing HPV16 and HPV18 probes. The standard protocol was modified to include prehybridization. The samples used were three batches of cytospun cells. The blinded study was completed using these samples as follows: six samples, labeled as one through six, were shown to an independent observer. Observer scored each sample by comparison with the others. All of the samples were correctly scored, positive results were scored as positive and the converse. In addition, the different samples were correctly scored for cell type. Therefore, there is a clear difference between signal and background when assessing bDNA in situ hybridization of hpv16 and hpv18 probes on both SiHa and HeLa cells, such that the positive result is obvious when compared to the negative result. In addition, cells grown on chamber slides were also subjected to hpv16 and hpv18 hybridization, but these samples were not included in the blinded study.

SIGNED BY _____ DATE _____

WITNESSED AND UNDERSTOOD BY _____ DATE _____

CROSS REFERENCES:

72

BAYER CORPORATION

SUBJECT bDNA in Situ

Consensus DNA protocol. August 25, 1999
For the detection of a DNA target.
Specimens for this protocol: cytospun cells, cells cultured on chamber slides, cryosections of cell pellets.

1. Rehydrate in Etoh. PBS wash2x2'.
2. 40 ug/ml RNase in 2x ssc 37 1h. 160 ul 10mg/ml per 40 ml.
3. PBS wash 2x2'.
4. 7.5 ug/ml proteinase K in PBS, 37C for 10'.
   15 ul 20 mg/ml per 40 ml.
5. PBS wash 2x2'. Dehydrate.
5b. Prehybridization for 30' RT.
6. Denaturation in prehyb at 92C for 5'.
6b. Optional. Wash in PBS. Dehydrate. air dry.
7. Replace with fresh hyb (containing probe). Hyb 40C for 3h for SiHa or HeLa samples in 1st hyb buffer.
8. Wash: 2x SSC 1'x2; .2xSSC 1'x2; 0.1xSSC 5'. With 0.0025% Brij (Deteregent): 2.5 ul 10%Brij/ 10 ml wash. rt.
9. hyb 25'@55c in 2nd hyb, which is Cao 2nd hyb, with 0.9 fmol/ul probe.
10. Wash in buffer 2 (0.1xSSC, 1mM EDTA) for 10", 1' and 4'.
11. Repeat for amp.
12. hyb for 15' at 45 with 0.9 fmol/ul AP probe in 3rd hyb.
13. Wash in HIV wash D. 3x as above.
14. AP Development,10' at RT.

1st hyb buffer	1.5 ml		0.8 ml
50% formamide	.75 ml		0.4
10% Dex Sul	.37 ml	40%	0.2
0.2% Casein	30 ul	10%	16 ul
3x SSC	.225 ml	20X	0.12
10 ug/ml polyA	1.5 ul	10 mg/ml	0.8
100 ug/ml ssDNA	15 ul	100 mg/ml	8
5 fmol/ul probe (1/40)	31 ul	200 fmol/ul	
	1,391 +109 h2o		.745 add 20 ul probe 35 ul water

2nd hyb buffer	4	1 ml	1.5 ml
5x SSC	1	250 ul	375 ul 20X SSC
0.5% Casein	200	50 ul	75 ul 10x/10%
0.3% SDS	120	30 ul	45 ul 10% SDS
10% DexSul	1	250 ul	250 ul 40% Dex Sul
0.9 fmol/ul probe			
	2.32 + 1.68 H2O		

SIGNED BY _____ DATE _____

WITNESSED AND UNDERSTOOD BY _____ DATE _____

CROSS REFERENCES:

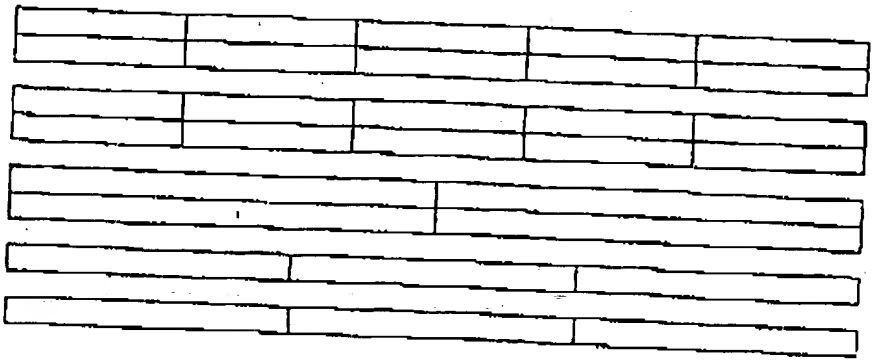
BAYER CORPORATION

73

SUBJECT EDNA In Situ

3rd hyb buffer	1ml
5xSSC	250 ul 20x
0.5% Casein	50 ul
0.3% SDS	30 ul
10% DexSulf	250 ul 40% Dex Sulf
1mM ZnCl2	10 ul 100mM
10 MgCl2	20 ul 0.5 MgCl2

qs to 1ml with 390 ul water.



1st hyb	3ml
form	1500 ul
Dex	740
Case	60
ssc	450
polya	3
ssdna	30
	2783
water	142
probes	75

3rd hyb	2ml
ssc	500 ul
case	100
sds	60
dex	500
Znci	20
Macl	40
	1220
water	780

SIGNED BY _____ DATE _____

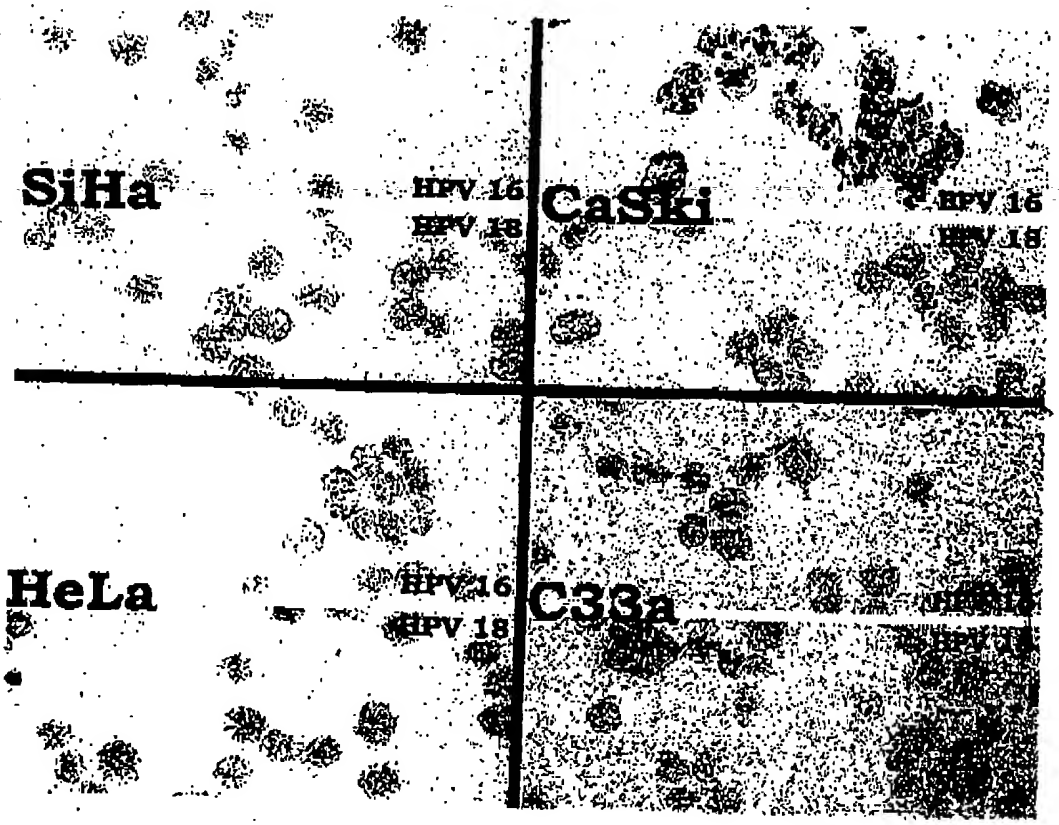
WITNESSED AND UNDERSTOOD BY _____ DATE _____

CROSS REFERENCES:

86

BAYER CORPORATION

SUBJECT: b-DNA In Situ



SIGNED BY Dawn Kenyon DATE

WITNESSED AND UNDERSTOOD BY DATE

CROSS REFERENCES:

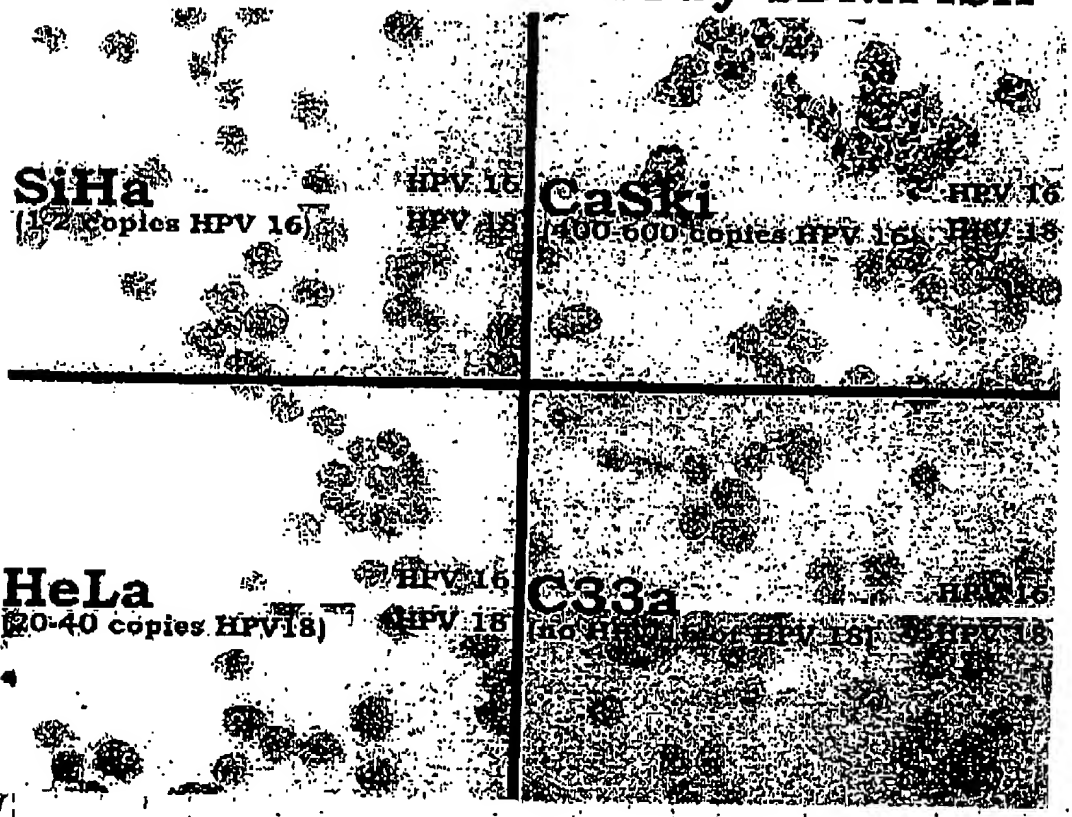
92

BAYER CORPORATION

SUBJECT b DNA In Situ

Survey of cell lines for HPV DNA

Detection of HPV DNA by bDNA ISH



27
28
29
30
31
32
33
34

SIGNED BY Dawn Kemp DATE

WITNESSED AND UNDERSTOOD BY DATE

CROSS REFERENCES:

BAYER CORPORATION

99

SUBJECT bDNA In Situ

Branched DNA in situ hybridizatio

- Sensitive
  - Single copy gene detection
- Specific
  - Distinguishes HPV genotypes
- Localized
  - Colocalization of signal and target
- Versatile
  - DNA and mRNA detection
  - Chromogenic and fluorescent signal
  - Applicable to automation
  - Quantitative
- Convenient
  - Rapid (within one day)
  - Nonisotopic

27
28
29
30
31
32
33
34

SIGNED BY _____ DATE

WITNESSED AND UNDERSTOOD BY _____ DATE

CROSS REFERENCES: